MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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I. Introduction

During this quarter studies continued which mapped sites in the sacral spinal cord which produce changes in cavernous sinus pressure to microstimulation. Recording of cavernous pressure from the cat penis seems to be a good model for quantification of penile erection in the anesthetized male cat. Tracing studies using pseudorabies virus and concerned with determining the location and distribution of penile efferent and interneurons also continued during this quarter.

In addition during this quarter new experiments were begun which examined the motor responses of flexor and extensor muscles of the knee joint to microstimulation of the lumbosacral spinal cord. Tracing studies complimenting these microstimulation studies were also initiated this quarter. This progress report will provide a detailed discussion of the new experiments conducted this quarter and concerned with motor activity of the hindlimb.

II. Extension and Flexion of the Hindlimb at the Knee Joint to Microstimulation of the Spinal Cord.

A. Introduction

The purpose of these studies are to determine: (1) the feasibility of controlling lower hindlimb flexion or extension by electrical stimulation of the lumbosacral spinal cord with fine tipped microelectrodes; (2) to map the lumbosacral spinal cord using microstimulation to determine sites which produce extension or flexion of the shank (part of the hindlimb below the knee), and (3) to determine, using tracing techniques, the location and distribution of efferents and interneurons which innervate and control the flexors and extensors of the shank. The tracing

studies would provide important sites for microstimulation and would also aid in the interpretation of the stimulus data.

The long term goals of these studies are to produce fictive leg movements by microstimulation of the spinal cord in human patients following upper spinal cord injury.

B. Methods

Adult cats, weighing 3.1 to 4.5 kg were used in these studies. The animals were initially anesthetized with halothane (3%): oxygen and following surgery were anesthetized with pentobarbital (30mg/kg iv) and supplemented with pentobarbital as necessary throughout the experiment. Surgical procedures include: (1) placing a catheter into the right common carotid artery for blood pressure recording, (2) placement of an endotracheal cannulae to keep the airway patent and for artificial respiration when necessary, (3) placement of a catheter into the cephalic vein in the lower arm for drug and anesthetic administration, (4) a catheter is also placed transurethrally to empty bladder and in some experiments to record bladder pressure together with motor activity, (5) a large laminectomy is performed to expose the L_4 to S_2 segments of the spinal cord and the dorsal and ventral roots, (6) the dura is opened and the roots and spinal segments identified. Suture loops are placed loosely around the S_1 to L_5 ventral roots for stimulation during the experiment. One or two dorsal roots, usually L_6 and L_7, are identified and loose ligatures placed around them for identification of the segments for stimulation with microelectrodes. (7) an adjustable aluminum bracket is attached to the tibia via two small stainless steel bolts. The aluminum bracket and the hip pins of the spinal frame secure the leg in a fixed position . The aluminum bracket attached to the tibia accepts a rotational torque sensor (Eaton-Lebow) for determining isometric torque about the knee joint for either flexion or

extension.

Following these surgical procedures the animal is placed in an Eccles' spinal frame. The spinal frame suspends the animal via hip pins, a lumbar spinal clamp, and a clamp on the dorsal spinous process at the thoracic level. This spinal apparatus rigidly fixes and prevents movement of spinal cord.

The skin around the laminectomy is sutured to the spinal frame to form a pool which is continuously perfused with warm (96-99° F) oxygenated Krebs solution. This protects the exposed spinal cord and surrounding muscle from drying and cooling.

The output signals from the blood pressure transducer, the torque sensor, and bladder pressure transducers are amplified, recorded on tape, displayed on a chart recorder, and also sent to a laboratory computer (IBM compatible PC) equipped with and A to D data acquisition board and Lab View Software for on-line processing and storage.

Hook electrodes were used to stimulate ventral roots while fine tip (200-400 u^2 exposed surface) activated iridium microelectrodes were used for microstimulation of the spinal cord. Stimulations were constant current negative first bipolar pulses. The stimulus parameter varied from 10-150uA, 10-100Hz, 0.05 to 0.4msec duration, (30 sec "on", 2 min "off").

The spinal cord is mapped by making sequential electrode tracts every 200 or 300u across the spinal cord in a mediolateral direction. Each tract maps 3 to 4mm of spinal cord in the dorsoventral direction starting at the surface of the cord and moving deeper in 200u increments. At the end of an experiment the tissue is fixed with formalin and examined histologically for position of electrode tracts. The electrode position and response at that level of the spinal cord are then correlated.

C. Results

Prior to microstimulation of the spinal cord the response to ventral root stimulation is recorded. The response to ventral root stimulation is important for two reasons. First, these responses give us an indication of the magnitude and type of response (flexion or extension) we may expect from this particular segment of the spinal cord when compared to microstimulation. Second, by determining the ventral root response early in the experiment, any deterioration in this response seen over time would indicate that the peripheral component of the pathway is the likely site of damage and not the site of microstimulation.

Figures 1 and 2 are examples of the torque generated by stimulation of the S_1, L_7, L_6, or L_5 ventral root at increasing intensities. Notice that S_1 and L_7 ventral root stimulation produces primarily a flexor response while L_6 and L_5 produce extension. It should also be noted, although not shown in Figures 1 and 2, that a variety of other responses occur with ventral root stimulation such as foot and toe extension and flexion. This is especially true of L_7 which innervates many muscles of the leg, foot and ankle.

It should also be noted (see Figures 1& 2) that in some instances (particularly at the higher intensities of stimulation) fatigue of the response to ventral root stimulation occurs. This is likely a property of the muscle and neuromuscular junction and is seen with microstimulation of the spinal cord as well.

Spinal cord stimulation with fine tipped microelectrodes produced a somewhat weaker response than that seen with ventral root stimulation. The responses varied depending on the location in the spinal cord, the segment, the depth, and the mediolateral position. All were important in determining response of the knee joint. Most of our initial experiments were

performed at the L_5 and L_6 levels of the spinal cord. These segments produce extension wi. ventral root stimulation. Figure 3 A, B, C, and D show some responses to microstimulation at various depths from the surface of the L_6 cord at four progressively (200u increments) more lateral distances from the initial penetration(tract A in Fig 3). Tract A is located just medial to the dorsal root entry zone (DREZ) while B, C, and D are 200, 400, and 600u lateral to A respectively. Notice in figure 3A that flexion occurs with stimulation of the dorsal horn (depth 1.0 to 2.0mm) and there is very little response in the medial part of the ventral horn (depth 2.4 to 3.4mm). These small flexor responses seen in the superficial spinal cord are likely due to reflexes produced by afferent stimulation in the dorsal horn. The extensor response is best produced near the motor neurons in the ventral horn especially where the axons of these neurons accumulate to form small bundles at the base of the ventral gray matter. The responses seen with the microelectrode tip located deep in the ventral gray matter is often quite large. Good extension response can often be produced in L_5 and L_6 with little ankle, foot and toe movement. As intensity of stimulation is increased however, more foot and ankle movement is seen. Although L_7 was examined in only one experiment it seems more difficult to activate the flexor muscles in the absence of other hindlimb movements.

The parameters of stimulation for both the spinal cord and ventral root were important in determining the amplitude, the amount of fatigue, the smoothness of the extensor and flexor responses. Figure 4 shows the effects of varying the intensity (top of Figure 4) and frequency (bottom of Figure 4) of the stimulus for spinal cord microstimulation at the L_6 level.

Notice that as the intensity of the stimulus is increased the maximum torque for extension is also increased. Our mapping stimulus is usually 50 to 100uA and at these intensities a good

response is usually seen without many nonspecific responses. Few responses are seen below 50uA.

Changes in stimulus frequency is also an important parameter for producing maximum torque with few nonspecific effects or fatigue is seen at about 40Hz (see Figure 4, bottom). Torque increases with increasing frequency to about 35 to 50Hz. Also, at higher frequency especially above 50Hz the response shows considerable fatigue (not shown in Figure 4). Fatigue is the drop in torque over the duration of stimulation. As the frequency is lowered the torque generated drops, and at 10 to 15Hz individual muscle twitches are seen and the torque curve becomes jagged. During our mapping experiments a frequency of 40Hz is usually used.

During the next quarter these various types of experiments will continue and include a more detailed examination of the motor responses of the S_1 and L_5 segments.

Figure 1 Plots of the torque generated about the knee joint to stimulation of the S_1 (to, L_7 (bottom) ventral roots. Arrows indicate the onset and offset of root stimulatic for a 30sec period. An upward deflection is flexion of the knee joint while downward deflection is extension. The smallest stimulus intensity (1V) produces no change in torque while the largest (20V) produces the highest amplitude torque. Intensities are 1, 2, 3, 4, 5, 7.5, 10, 15, and 20 volts. The other stimulus parameters are 0.05 msec duration pulses at 40 Hz for 30 seconds "on" and 120 seconds "off". Notice that both S_1 and L_7 ventral root stimulation produces flexion at the knee joint and that with time fatigue occurs. Torque is measured in newton-meters (Nm).

Figure 2 Same as Figure 1 except that the L_5 (top) and L_3 (bottom) ventral root is stimulated and extension is produced by increasing intensity of stimulation from 1 to 20 volts.

Figure 3 Plots of the maximum torque generated about the knee joint by microstimulation of the L_5 spinal cord at different depths and four (A, B, C, and D) different areas (along a mediolateral line) of the L_5 cord. A is the most medial tract and is located just medial to the "dorsal root entry zone" (DREZ), while B, C, and D are each 200 u further lateral to the other. Depths are in mm from the surface of the cord. A small amount of flexion is seen in the dorsal horn while extension predominates in the ventral horn. Extension is plotted as negative torque while flexion is plotted as positive. Torque is measured in newton-centimeters. Stimulus parameters are: 100uA, 0.2msec duration, 40Hz, for 30sec "on" & 120sec "off." Each point is the peak torque during a 30 second stimulation. Notice that strong extension occurs with ventral horn stimulation at sties lateral to the DREZ.

Figure 4 Plots of the maximum torque generated about the knee joint by microstimulation

of the L_4 cord to increasing intensities (top) and frequencies (bottom) of stimulation. The L_4 cord is activated by a microelectrode whose tip is 2.6mm from the cord surface. The stimulus parameters for the plot at top are: 0.2msec duration, 40Hz, 25 to 150uA for 30sec. "on" & 120sec. "off." The stimulus parameters for bottom plot are: 0.2msec duration, 100uA, 10 to 100Hz, 30sec. "on" & 120sec. "off". Peak torque during 30 second stimulus is plotted. Notice that the peak torque is generated at a frequency of 40 to 50 Hz. The maximum intensity tested was 150uA.

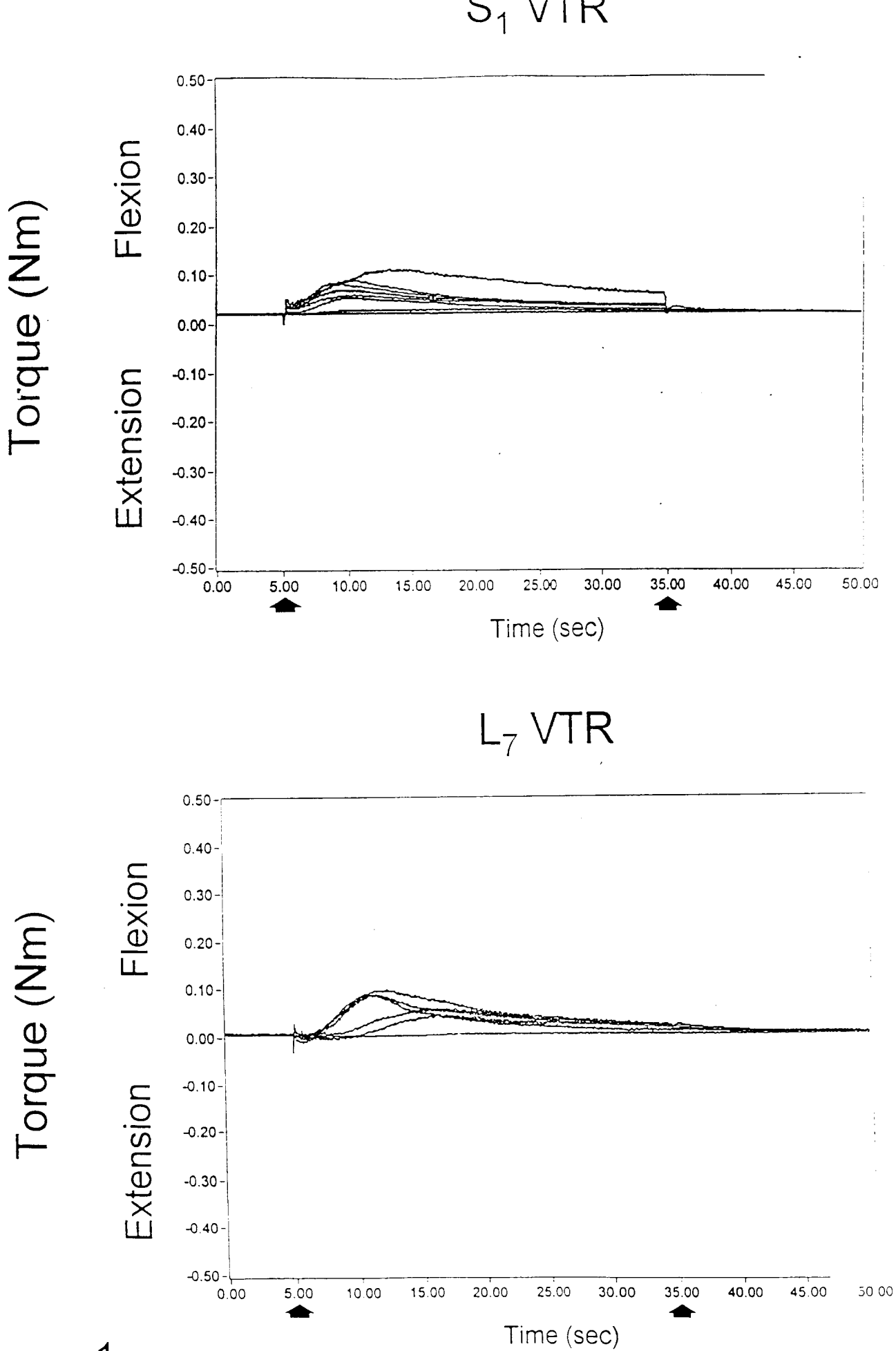

S_1 VTR
Torque (Nm)
Flexion
Extension
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Time (sec)
L_7 VTR
Torque (Nm)
Flexion
Extension
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Time (sec)

Figure 1

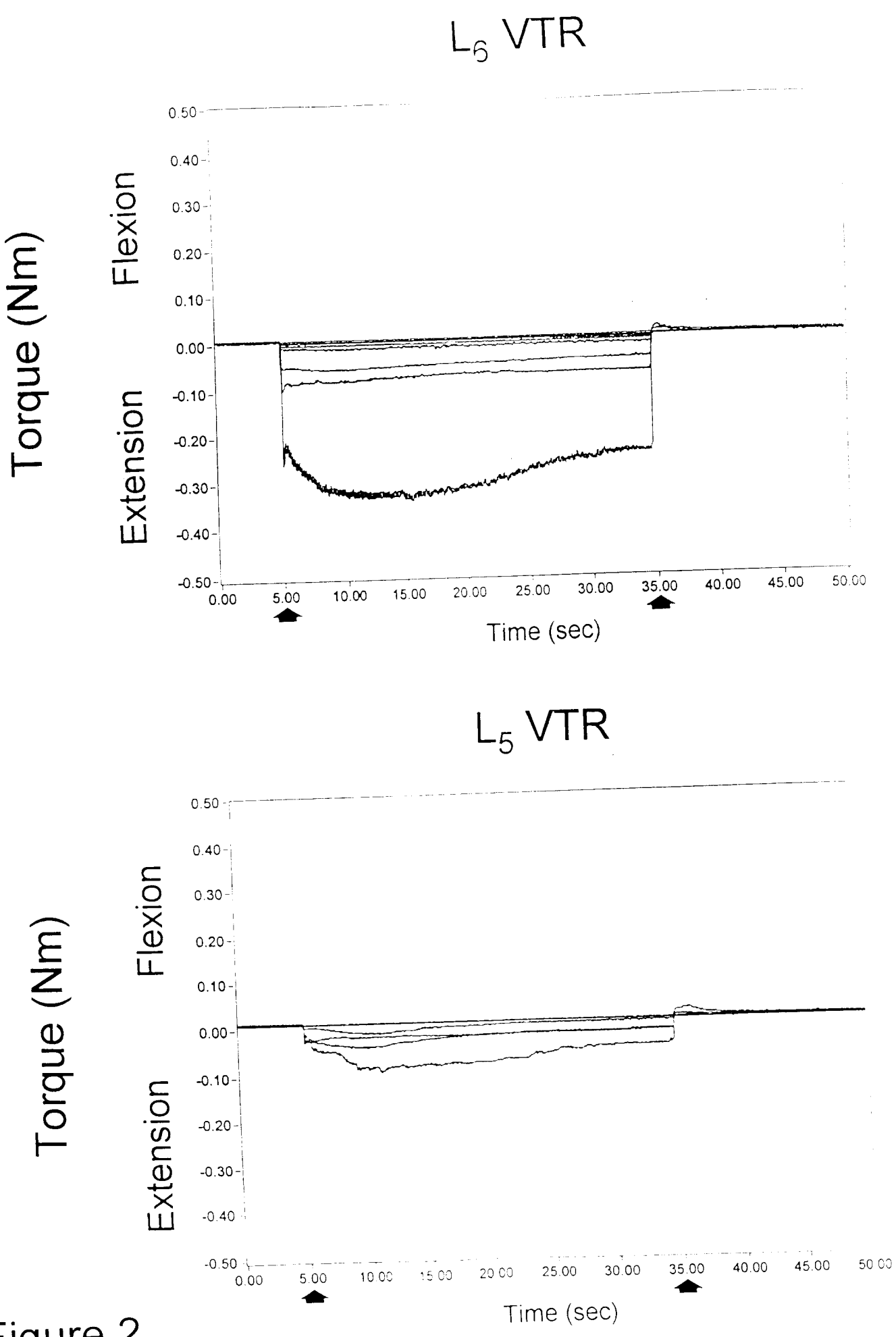
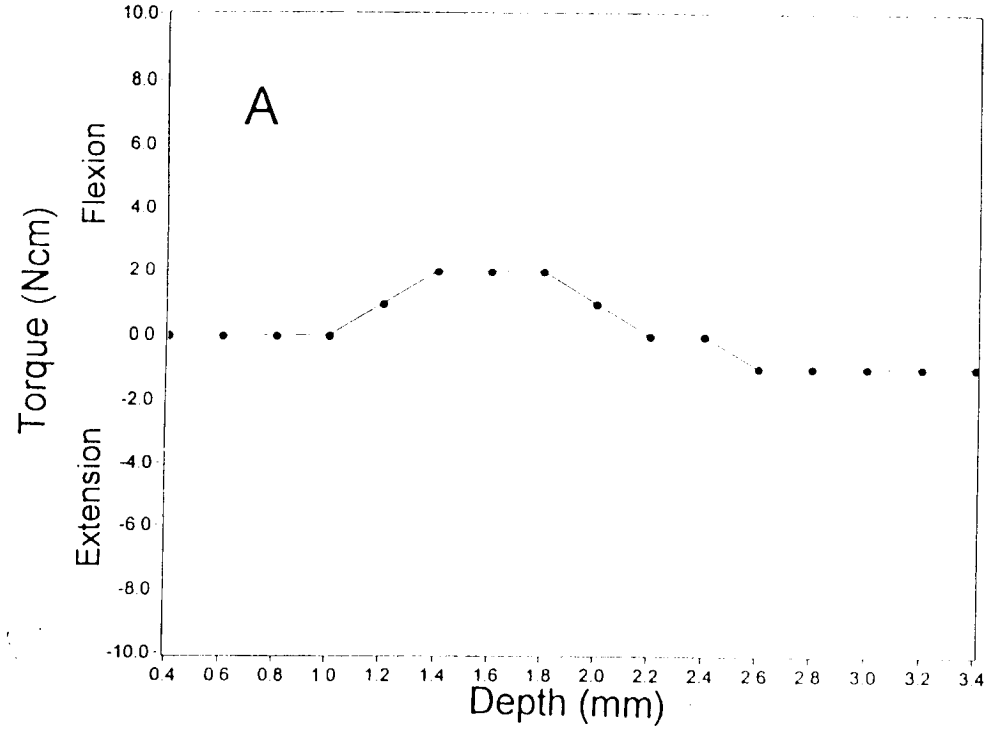
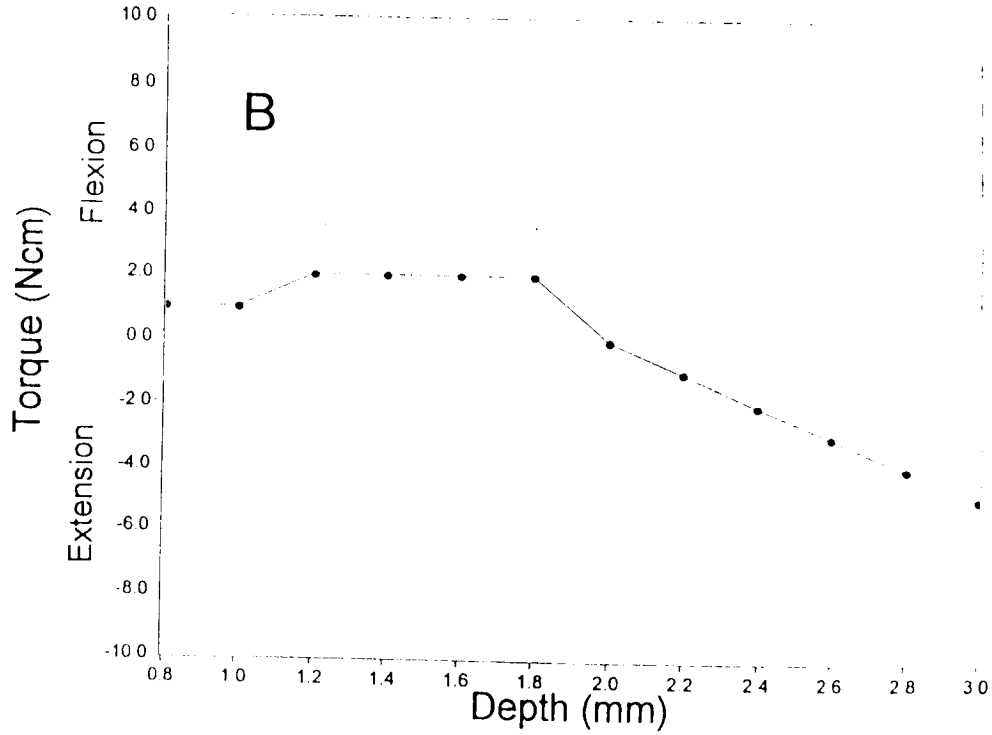
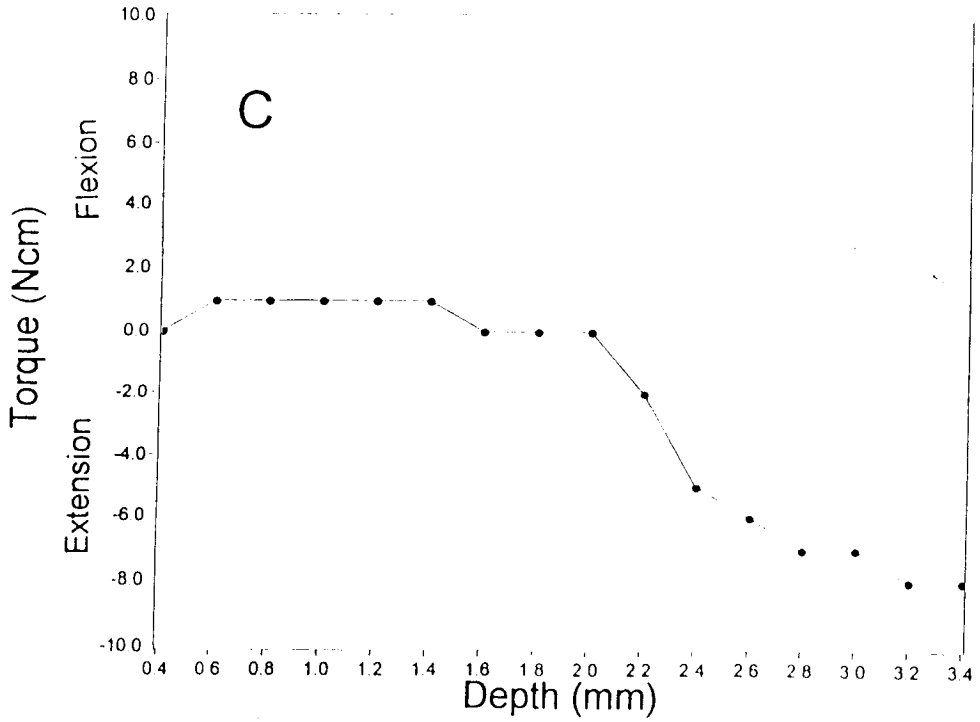

L6 VTR
Torque (Nm)
Flexion
Extension
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Time (sec)
L5 VTR
Torque (Nm)
Flexion
Extension
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Time (sec)

Figure 2


A
Torque (Ncm)
Flexion
Extension
Depth (mm)


B
Torque (Ncm)
Flexion
Extension
Depth (mm)


C
Torque (Ncm)
Flexion
Extension
Depth (mm)

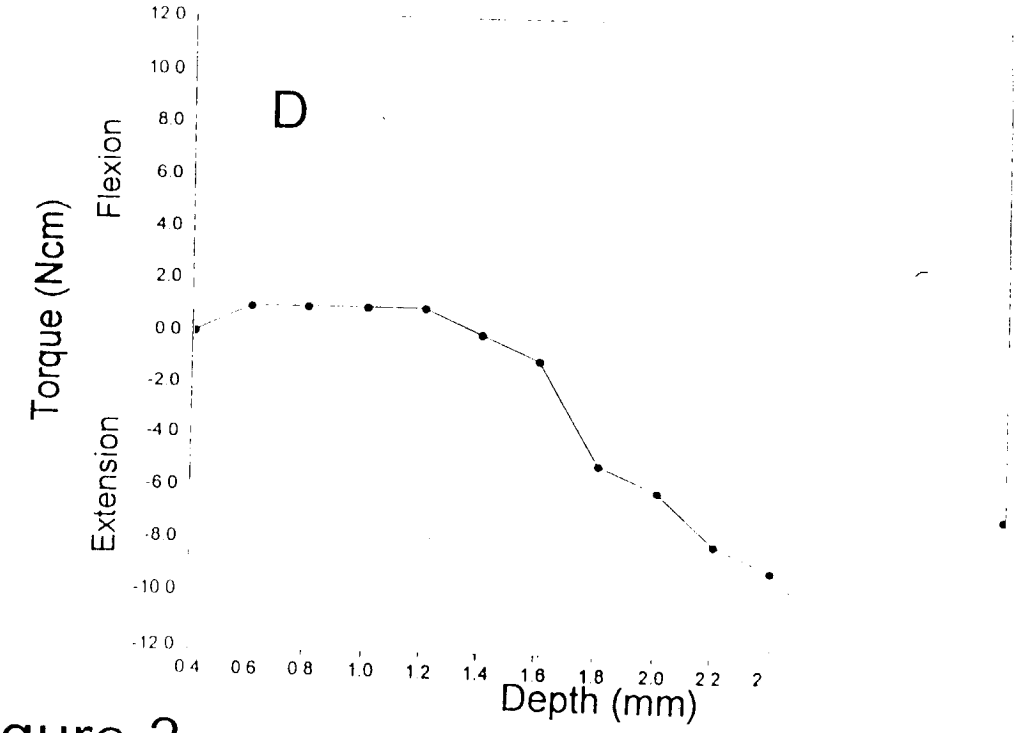
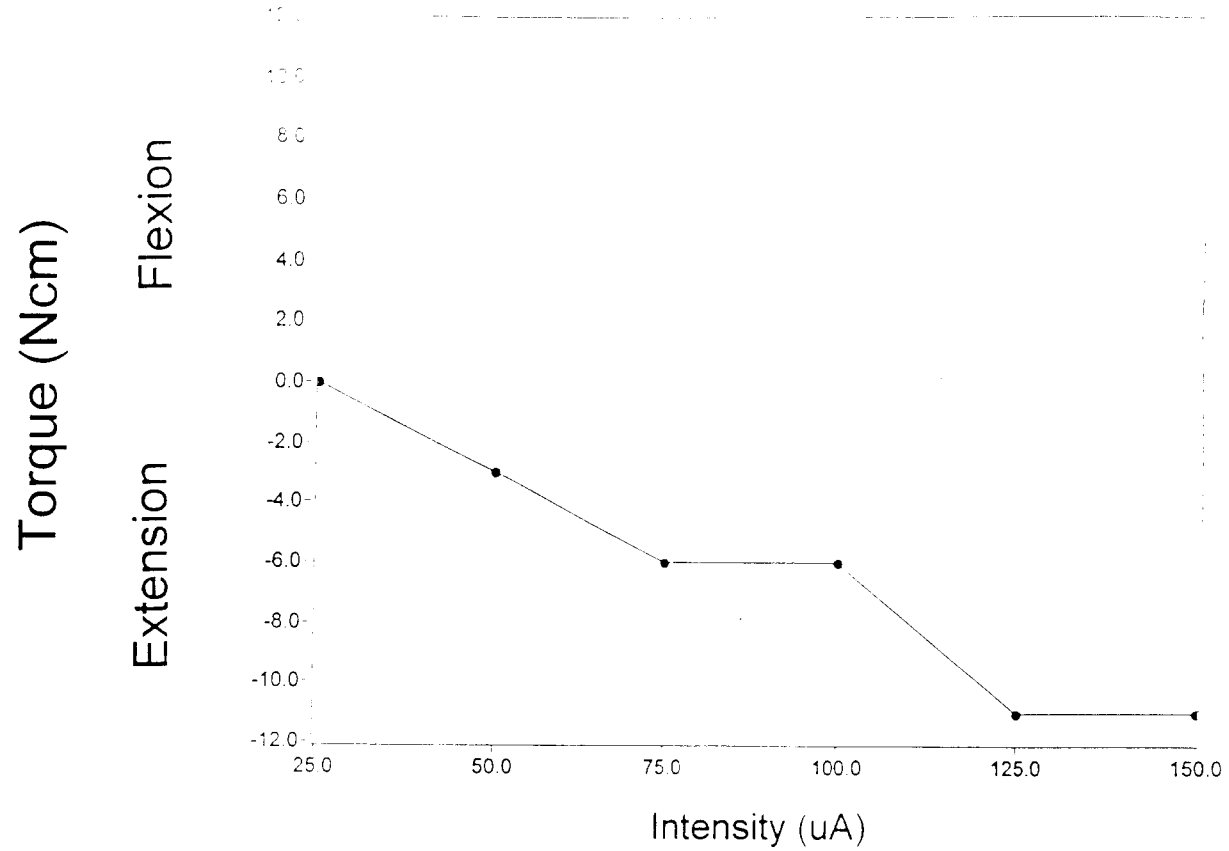

D
Torque (Ncm)
Flexion
Extension
Depth (mm)

Figure 3


Torque (Ncm)
Flexion
Extension
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
25.0
50.0
75.0
100.0
125.0
150.0
Intensity (uA)

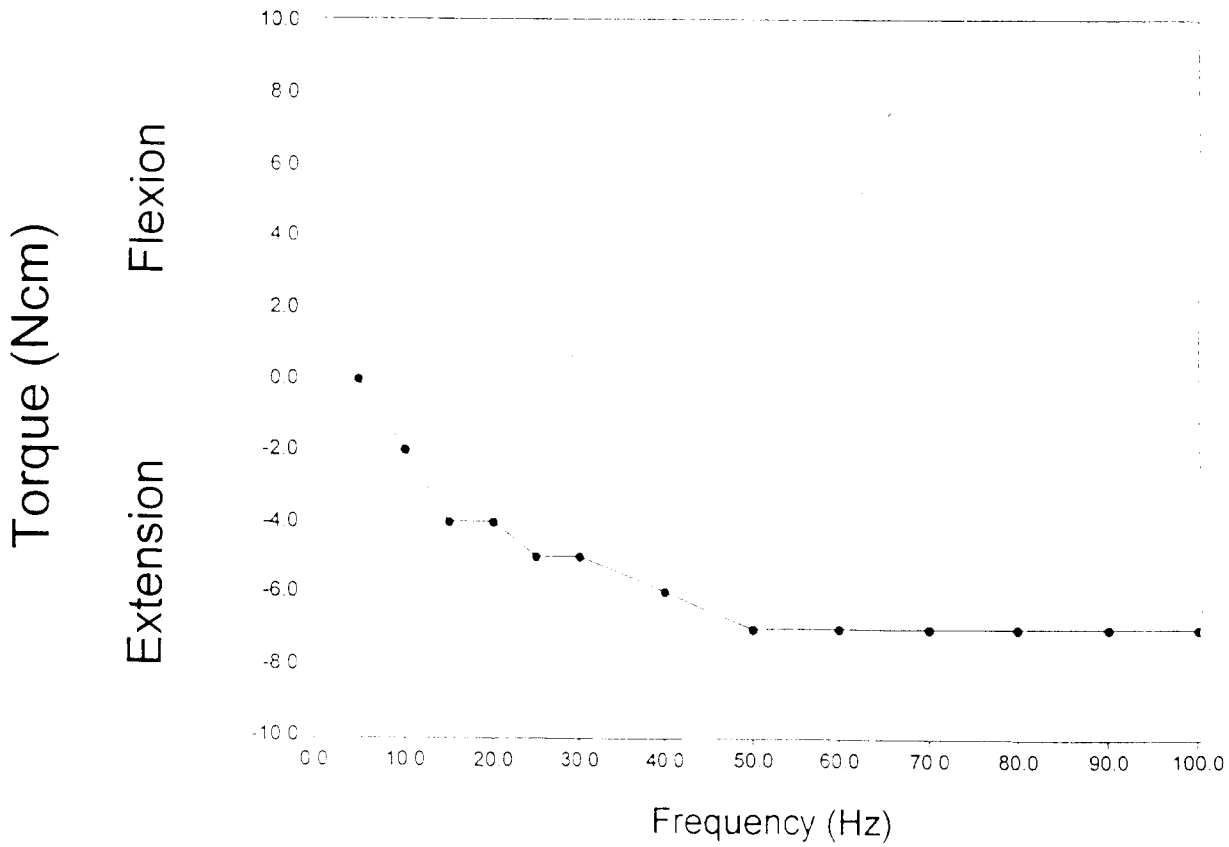

Torque (Ncm)
Flexion
Extension
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
Frequency (Hz)

Figure 4